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Chapter 1

General Introduction

Bone defects can lead to a decrease in the overall health and life quality of patients, and the need for bone regeneration is increasing dramatically as the world's population ages [1, 2]. Natural bone tissue has a certain regenerative capacity; however, it is too limited to repair large-volume bone defects. Furthermore, the limited regenerative capacity of bone tissue can be further significantly compromised by many adverse local and systemic conditions, such as alcoholism, osteoporosis, and bone infections [3-6]. In the clinic, these compromised regenerative capacities can hinder the repair of (large) bone defects, delay the osseointegration of dental implants, and increase the risk of bone fracture. This situation usually causes severe pain, reduces the quality of life, and imposes an economic burden on the patient and on society. Hitherto, in the fields of implantology, maxillofacial surgery, and orthopedics, it has been challenging to repair bone defects in adverse conditions; thus, the development of effective treatment options to repair bone defects in adverse bone conditions is urgently required.

Bone defect in adverse conditions

1. Alcoholism

One of the most common adverse conditions for bone repair is alcoholism. About 202.3 million people (age ≥ 15) (4.1%) worldwide have alcohol use disorders in 2010 [7]. The detrimental effect of alcohol is more harmful than nicotine [8]. Epidemiological evidence indicates that chronic alcohol abuse leads to low bone mass [9-13], bone fragility, and spontaneous fractures [9, 14-16]. Animal studies also show that alcohol consumption can significantly decrease new bone formation [17] and delay the osseointegration of implants [18], at least partially by reducing the number and activity of osteoblasts [19]. Alcoholism can also result in compromised osteoinduction, thus leading to impaired healing of bone defects [19]. Furthermore, prenatal alcohol exposure also significantly affects fetal bone development [20]. Chronic alcohol abuse significantly increases the concentration of all-trans retinoic acid (ATRA) [21], a metabolite of alcohol, which might account for the detrimental effects of alcoholism [22]. ATRA can significantly compromise osteoblastogenesis [23]. Therefore, ATRA is regarded as a key mediator for the suppression of bone regenerative capacity in alcoholic patients.

2. Osteoporosis

Osteoporosis has become an alarming health problem worldwide. It is often described as a silent disease because it is typically asymptomatic. Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and fracture risk. When a fracture occurs, the disease negatively and significantly affects morbidity and mortality, because it can lead to severe pain, deformity, disability, and death [24]. About 200 million people worldwide are under the threat of osteoporosis [25]. In recent decades, several drugs have been developed to cure osteoporosis; however, their effects are less than satisfactory. Therefore, developing better treatments is of paramount importance.

3. Infected bone defects and peri-implant infection

Another adverse condition for bone repair is infection. Infected bone defects can result from acute high-energy injuries and chronic infectious diseases. According to the World Health Organization, injuries are a global public health problem [26]. There are approximately 3–9 million injuries recorded annually in developed countries. The number of deaths from injuries has doubled in the last 30 years in European countries [27]. High-energy injury to the extremities can result in open comminuted fractures that easily develop into severe osteomyelitis when a bacterial infection is introduced because of compromised immune protection. Osteomyelitis is difficult

to completely eliminate and remains a formidable medical problem, particularly when it accompanies large bone defects. *Staphylococcus aureus* is the main bacterial pathogen, accounting for 80% of human osteomyelitis [28].

It can also be caused by chronic bacterial infections, such as periodontitis and peri-implantitis, which are diseases surrounding natural teeth and dental implants with the loss of supporting bone through a bacterial etiology. The prevalence of severe periodontitis with deep periodontal pockets (≥ 6 mm) ranges from 10% to 15% in adults [29]. The incidence of peri-implantitis is 8.6 to 9.7% after 5 years [30, 31] and as high as 14% after 10 to 15 years [32]. Peri-implantitis exhibits a more pronounced infection progress in the mucosa compared with periodontitis [33]. In addition, the infected lesion of peri-implantitis extends more rapidly into the bone marrow, leading to a greater extent of bone loss compared with periodontitis [34, 35]. Peri-implant infection remains a major challenge for proper implant fixation and durability [36-39]. However, the effectiveness of current therapeutic approaches to prevent peri-implant infection is not satisfactory.

Bioactive reagents to treat bone defects in adverse conditions

1. Osteoinductive growth factors: homodimeric and heterodimeric bone morphogenetic proteins (BMPs)

To promote bone defect repair, bone tissue engineering including gene, cell, and cytokine therapies, has been developed since the last decade. Among the three therapies, cytokine therapy is advantageous in terms of safety, feasibility, and its potential for clinical application compared with the other two. Growth factors, as an important cytokine family, play an important role in wound healing and tissue regeneration. They are naturally occurring proteins that are essential in cellular signaling for growth, proliferation, and differentiation [40]. To promote bone regeneration to repair bone defects, one strategy could be the adoption of potent osteoinductive growth factors, such as BMPs, a group of disulfide bond-linked dimeric proteins of the transforming growth factor- β superfamily. Most mature BMP molecules consist of two monomers that are covalently linked via a disulfide bond [41, 42]. When the two monomers in one ligand are derived from the same BMP member, the BMP ligand is termed “homodimeric BMP” or a BMP homodimer. In the USA, Europe and Australia, homodimeric BMP2 and BMP7 have already been approved for clinical application in nonunion bone fractures and spinal fusions [40, 43]. However, the effective doses of homodimeric BMPs in the clinic that promote bone formation are extremely high (e.g., up to milligrams) [44, 45], which results not only in a substantial economic burden to patients, but also a series of potential side effects, such as the overstimulation of osteoclastic activity and ectopic bone formation in an unintended area [46, 47]. Thus, researchers have turned to “heterodimeric BMP” or BMP heterodimers, in which the two monomers are derived from different BMP members. Compared with BMP2 and BMP7 homodimers, the BMP2/7 heterodimer induced osteoblastogenesis at a significantly lower threshold concentration, but with similar maximum effects. Animal experiments also verified this result, and the structure of the new bones induced by BMP2/7 was more similar to normal bone [48]. The function of ATRA on bone metabolism can be mediated by decreasing osteoblastic bone formation [49]. Therefore, heterodimeric BMP2/7 could be a good strategy to promote bone regeneration to repair bone defects in patients with alcoholism. **In chapter 2**, we therefore compared the efficacy of heterodimeric BMP2/7 and homodimeric BMP2 and BMP7 in restoring osteoblastogenesis under inhibition of ATRA. Bone defect repair is a process in which small amounts of bone are resorbed by the activity of osteoclasts, followed by the recruitment of osteoblast precursors that differentiate and replace the amount of resorbed bone [50]. Bone resorption by osteoclasts has to be matched with the generation of osteoblasts from precursor cells to replace the resorbed bone [51]. Thus, **in chapter 3**, we

compared the function of heterodimeric BMP2/7 and homodimeric BMP2 in osteoclastogenesis under exposure to ATRA.

2. Traditional Chinese medicine: Notoginsenoside R1 (NGR1)

Cytokine therapies like BMPs have been reported to substitute autografting; however, because of their high cost, they have not been widely used clinically [52-54]. In addition, we have found that BMP2/7 has promotive efficiency not only on osteoblastogenesis, but also on osteoclastogenesis [55, 56], which might be not suitable to repair bone defects with overstimulated osteoclastic activities, such as osteoporosis. In comparison, Traditional Chinese Medicine has played an important role in the prevention and treatment of osteoporosis in China for centuries. One such Traditional Chinese Medicine is *Panax notoginseng* saponins (PNS), a mixture of active compounds that are extracted from the *Panax notoginseng* root. *Panax notoginseng* has been widely used to treat osteoporosis for thousands of years in China and exhibits minimal side effects, which is a great advantage [57-60]. Recent studies suggested that PNS might be a potential therapeutic method to treat bone nonunion, osteoporosis, and osteonecrosis through the transforming growth factor-beta 1 (TGF- β 1) signaling pathway [61, 62]. Notoginsenoside R1 (NGR1) is one of the main constituents of PNS. Unlike other pharmacologically active saponins in both PNS and other species of ginseng, NGR1 is found only in PNS [63, 64]. Therefore, further research should be performed to evaluate the osteoinductivity of NGR1. **In chapter 4**, the function of NGR1 in the osteoblastogenesis of a pre-osteoblast cell line (MC3T3-E1) was explored in an *in vitro* time-course and dose-dependence study.

3. Antibacterial agents: HACC and AgNPs

To provide a viable treatment option for infected bone defects, continuous attempts have been made to antagonize infections. Antimicrobials applied locally or adsorbed onto implant surfaces cannot be sustained for long periods because of burst release [65-67]. Antibiotics usually have a narrow antibacterial spectrum. The long-term local administration of antibiotics may also cause antibiotic resistance. A novel water-soluble chitosan derivative (hydroxypropyltrimethyl ammonium chloride chitosan, HACC) has attracted much attention because of its strong antibacterial activity, broad spectrum [68, 69], and little reported bacterial resistance [70], which make it a promising candidate antibacterial biomaterial to treat infected bone defects [71]. It has been reported that HACC has antibacterial activity against both gram-positive and gram-negative bacteria [72]. **In chapter 5**, we used our previously developed osteoinductive bone substitute, combined with HACC, to develop a favorable local biomaterial system to treat infected critical-sized bone defects. **In chapter 6**, we reviewed the current knowledge concerning novel biomaterials with both antibacterial and osteoinductive properties to treat infected bone defects.

Another effective therapeutic approach for infected bone defect healing is the use of nanosilver/silver nanoparticles (AgNPs). AgNPs show a very promising application potential because of their broad antibacterial spectrum and lack of induction of resistance [73, 74]. AgNPs have already been successfully used in several medical applications, such as cardiovascular implants [75], central venous catheters [76], neurosurgical catheters [77], bone cements [78], and wound dressings [79]. During bacterial infection, the pH level around the peri-implant surface decreases to as low as pH 5.5 [80]. This change in pH can be used as a switch to control antimicrobial drug release from the implant surface. **In chapter 7**, we therefore aimed to design a pH-dependent AgNPs-releasing titanium nanotube array (TNT) implant for peri-implant infection control.

The aims of this thesis are as follows:

1. To investigate that whether heterodimeric BMP2/7 has advantages over homodimeric BMP2 or BMP7 in antagonizing the inhibitory effect of ATRA on osteoblastogenesis. We also investigated the molecular mechanism of the inhibitory effect of ATRA on osteoblastogenesis.
2. To reveal the antagonism of BMPs of different dimerization types and dose-deficiencies to ATRA-mediated inhibition of osteoclastogenesis, as well as to investigate the molecular mechanism underlying the inhibitory effect of ATRA on osteoclastogenesis.
3. To develop a new, single component for the treatment of bone defects, and to investigate the effects of NGR1 on the osteoblastogenesis of a pre-osteoblast cell line (MC3T3-E1) in an *in vitro* time-course and dose-dependency study.
4. To provide a viable treatment option for infected bone defects by developing a novel bone-defect-filling material with a sequential release system comprising the initial burst release of a powerful antibacterial agent-Hydroxypropyltrimethyl ammonium chloride chitosan (HACC), followed by the controlled release of BMP2.
5. To summarize the current knowledge concerning novel biomaterials with dual functions to treat infected bone defects, and to review those biomaterials with antibacterial and osteoinductive properties.
6. To design a low pH-triggered silver releasing TNT-AL-AgNPs implant to control peri-implant infection. We characterized the physicochemical properties of TNT-AL-AgNPs, analyzed the pH-dependent release of AgNPs from TNT-AL-AgNPs, and tested the antibacterial efficiency of the released AgNPs. We evaluated the biocompatibility of TNT-AL-AgNPs, as well as their effects on osteoblast morphology and differentiation *in vitro*.

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